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Maternal ingestion of locoweed II. The ability of intoxicated ewes to discriminate their own lamb

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Abstract

Consumption of locoweed (*Oxytropis sericea*) by sheep causes neurotoxicity, but specific behavioral effects have been given little attention. We examined the ability of ewes, exposed to locoweed during gestation and/or lactation, to discriminate their own from alien lambs. Ewes ($n=6$ per treatment group) were fed a locoweed/alfalfa pellet during (1) gestation (day 100–130) (LC) but not during lactation; (2) lactation (day 10–50 postpartum) (CL) but not gestation; (3) both gestation and lactation (LL); controls (CC) were fed alfalfa hay. Ewe behavior was examined in a two-lamb choice test on postpartum days 20, 40, and 50 (days 10, 30, and 40 of locoweed feeding). Locoweed feeding increased ($P<0.05$) serum AST activity and swainsonine concentration compared to controls, indicating moderate intoxication. Discrimination of lambs was not adversely affected by locoweed exposure. Locoweed intoxication during gestation decreased ($P=0.06$) ewe approach times to the first lamb, while locoweed exposure during lactation increased ($P<0.03$) approach times to the first lamb. Ewes not receiving locoweed during lactation (CC, LC) were initially (postpartum day 20) slower to approach their own lambs, but on days 40 and 50 had faster approach times to their own lambs compared to CL and LL ewes. Intoxicated ewes displayed an apparent locoweed-induced hypersensitivity and nervousness; these lactating ewes spent a greater percentage of time with their lambs after contact than did controls on postpartum day 50. If clinical signs of toxicity are not severe, culling intoxicated ewes for behavioral reasons may not be warranted.
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Keywords: Toxic plants; Sheep; Maternal behavior; Swainsonine; *Oxytropis*

1. Introduction

Locoweeds (*Astragalus* and *Oxytropis* spp.) are probably the most serious toxic plant problem in the western U.S. (Burrows and Tyrl, 2001) with annual losses in excess of US $10 million (U.S.;

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Nielsen, 1978). The *Astragalus* and *Oxytropis* genera are closely related (i.e., legume family), and grow together on many of the same rangelands. Both are called locoweeds because they contain the same toxin, the indolizidine alkaloid, swainsonine (Molyneux and James, 1982). Ingestion of either *Astragalus* or *Oxytropis* causes the same clinical signs (Stegelmeier et al., 1995). Swainsonine disrupts glycoprotein processing in cells, resulting in vacuolation and cell death (Elbein, 1989). Pregnant sheep often eat locoweed, and maternal locoweed ingestion results in fetal lesions and toxicosis in neonate lambs (Hartley and James, 1975; Stegelmeier et al., 1999). Neonate survival depends in large part on optimum interactions with the mother (Nowak et al., 2000). Visual and acoustic cues allow ewes to recognize lambs at a distance shortly after birth (Terrazas et al., 1999; Searby and Jouventin, 2003). Recognition of lambs is mediated in the central nervous system (CNS; Ferreira et al., 2003), whereas chronic locoweed intoxication results in numerous CNS lesions in sheep (Van Kampen and James, 1970; Pfister et al., 1996). Maternal locoweed ingestion results in small, weak lambs that are unable to move and suck normally (Pfister et al., 2006b). Further, ewe behavior at parturition is also impacted by locoweed intoxication, but in more subtle ways compared to their lambs (Pfister et al., 2006a). Thus, the objective of the present study was to determine if locoweed ingestion by ewes affects: (1) their ability to discriminate their own from an alien lamb at a distance in a two-choice test; (2) the approach time of ewes to lambs; (3) the amount of time ewes spend with their own lamb after initial contact. Our hypotheses were that intoxicated ewes would move more slowly, take a less direct path to their lambs, make more errors in discriminating their own from alien lambs compared with controls, and spend less time with their lambs after contact.

2. Materials and methods

2.1. Animals, treatments, and plant material

Experimental procedures in this study were essentially the same as detailed in Pfister et al. (2006a). Twenty-four lactating, primiparous Columbia-Targhee ewes were allocated to four treatments ($n = 6$): (1) locoweed fed during day 100–130 of gestation (LC), (2) locoweed fed during day 10–50 of lactation (CL), (3) locoweed fed during both gestation and lactation (LL) for the intervals given above, and (4) controls fed only alfalfa hay (CC). Ewes were individually housed and fed 3 kg/day of a 10% locoweed pellet (85% alfalfa hay and 5% molasses; air-dry weight), or alfalfa hay. Locoweed (*Oxytropis sericea*) was collected fresh from the Raft River mountains in northern Utah (N41°54′ W113°22′; 3016 m elevation), air-dried, and ground through a 2-mm screen before pelleting.

2.2. Testing procedure

Ewe behavior was measured using a two-lamb choice test on days 20, 40, and 50 post partum. Birth was considered as day 0. For ewes receiving locoweed during lactation (CL, LL), these test days corresponded to day 10, day 30, and day 40 of locoweed feeding. For each test, ewes were separated from their lambs, held in a pen (0.75 m × 1.5 m) for 15 min, and prevented from seeing into the 10 m × 7 m test arena. Alien lambs were selected from a large herd, and were closely matched with treatment lambs in terms of age, appearance, and sex. Both alien and own lambs were then placed individually in two wire-mesh cages 4-m apart and 9-m equidistant from the gate of the pen in which the ewe was being held. A grid with 1 m × 1 m sections was marked in the arena using painted stakes. Each ewe was released into the arena for 10 min, and the time and path to reach the lamb(s) were recorded in real time using a video camera. Lambs were switched to the opposite cage for each test. We timed each test from videotape using a stopwatch, and charted each ewe's pathway using the reference grid. A planimeter was used to trace the ewes' path from the chart to determine distance traveled to reach the first lamb. We also determined the percentage of time each ewe moved beyond 1 m from her own lamb's cage after initial contact for the duration of the 10-min test period.

2.3. Serum chemistry

Locoweed toxicity of ewes was related to serum concentrations of swainsonine and aspartate aminotransferase (AST) activity, an enzyme released from liver tissue as a result of the toxic insult (Burrows and

Tyrl, 2001). Methods for swainsonine determination followed Stegelmeier et al. (1995), and methods for AST analysis followed James et al. (1970).

2.4. Statistical analysis

The data were analyzed as a 2×2 factorial to examine ewe locoweed exposure during gestation and/or lactation. Since ewes were tested on various days post partum, a mixed model repeated measures analysis was done using SAS (1999). This same model was used to evaluate treatment differences in serum swainsonine and AST. To meet normality assumptions, data for time and distance traveled were transformed using log $(X+1)$. The data for percentage of time ewes spent away from their lamb after first contact were transformed by arcsine; data were normally distributed after transformations. The model included factors for gestation exposure, lactation exposure, gestation × lactation, trial day, and subsequent interactions. When a significant ($P \leq 0.10$) treatment × trial interaction was noted, the LSD procedure ($P \leq 0.1$) was used to determine treatment differences for each trial. Fisher's exact test was used to test for differences in number of errors made by ewes in discriminating their own lamb.

3. Results

3.1. AST activity and swainsonine concentration during lactation

Serum AST activity is index of the enzyme inhibition of swainsonine when locoweed is ingested. Locoweed ingestion by ewes during lactation (CL, LL) resulted in an increase in serum AST activity ($P < 0.001$) compared to controls (CC, LC; Fig. 1). Activity of AST peaked around the 15th day of locoweed intake (day 25 of lactation). Further consumption of locoweed did not increase AST activity. There were both treatment effects and treatment by day interactions. Ewes that had previously received locoweed during gestation, but not lactation (LC) showed some residual effects from locoweed, but locoweed withdrawal initiated a slow decrease in AST activity in the LC ewes. Activity of AST in LC ewes intoxicated during gestation was initially elevated

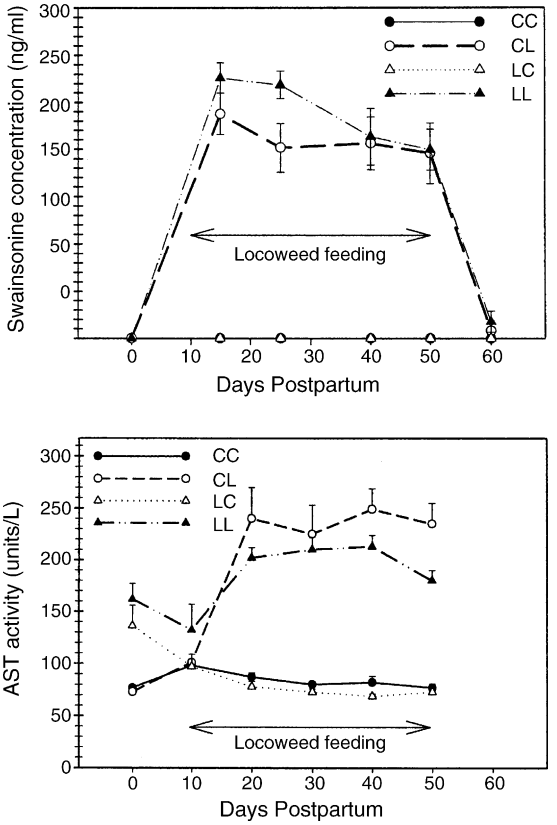

Fig. 1. Serum AST activity (unit/l) and swainsonine concentration (ng/ml) in ewes postpartum. Treatment groups include: CC—controls not fed locoweed during gestation or lactation; CL—ewes not fed locoweed during gestation, but fed locoweed during lactation day 10–50; LC—ewes fed locoweed from gestation day 100–130, but not fed locoweed during lactation; LL—ewes fed locoweed during both gestation and lactation.

compared to controls (CC), but by postpartum day 10 was not different than controls. Swainsonine was detected only in serum of ewes fed locoweed during lactation (Fig. 1), with rapid increases and decreases with initiation and withdrawal of locoweed feeding, respectively.

3.2. Ewe behavior in two-lamb choice-test

There was no effect ($P > 0.2$) of locoweed feeding on the number of errors made by ewes in discriminating their own lambs. Overall, the proportion of errors made by all ewes (i.e., failure to reach own lamb first) was 0.33. Locoweed consumption did not affect ewes'

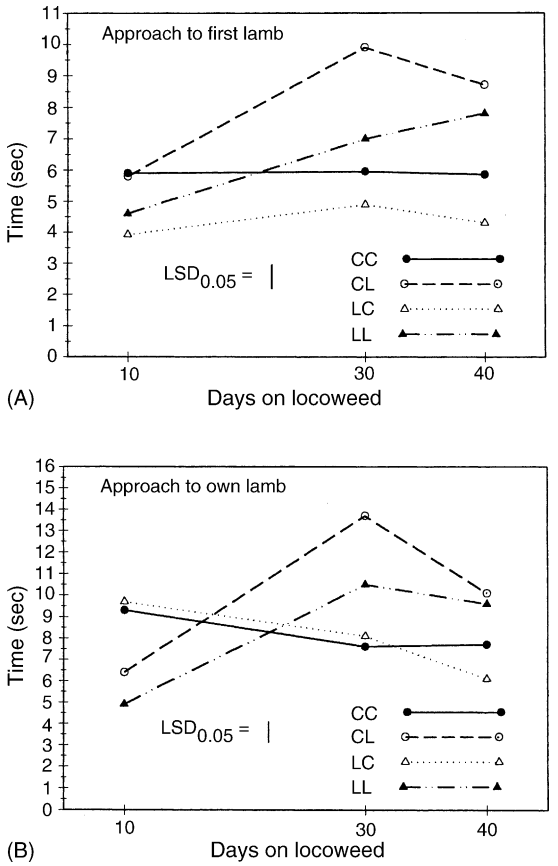

Fig. 2. (A) Approach time (s) for ewe to make first contact with a lamb (either alien or own) during the discrimination tests. (B) Approach time (s) for ewe to make first contact with it's own lamb during the discrimination tests. Ewes were tested after 10, 30 or 40 days of locoweed feeding, corresponding to postpartum day 20, day 40, and day 50. For statistical analysis, means were transformed by log $(X+1)$, but means shown here are in the original scale of measurement. Treatment groups are: CC—no locoweed during gestation or lactation; CL—no locoweed during gestation, but fed locoweed from day 10–50 of lactation; LC—locoweed fed from day 100–130 of gestation, but no locoweed during lactation; LL—locoweed fed during both gestation and lactation.

latency to reach their own lambs after making an initial mistake (data not shown).

Locoweed intoxication during gestation tended to decrease ($P=0.06$) ewe approach times to the first lamb, while locoweed exposure during lactation increased ($P<0.03$) approach times to the first lamb (Fig. 2A). There was no gestation × lactation exposure interaction, but a trial day × treatment interaction was noted ($P<0.01$). Locoweed exposure during gestation (LC), or 10 additional days of locoweed consumption during lactation (LL) decreased ($P=0.06$) ewe approach times to the first lamb (either alien or own) compared with controls (CC) or compared with ewes fed locoweed during lactation for 10 days (CL) (Fig. 2A). Further locoweed feeding during lactation increased ($P<0.04$) ewes' approach times to the first lamb. Ewes intoxicated during lactation (CL) were slower to approach the first lamb on day 30 compared to the other treatments, and on day 40 both lactation groups (CL, LL) were slower than were controls (CC) and the gestation-only group (LC).

There was a treatment × trial interaction ($P=0.04$) for ewes' approach times to their own lambs. Exposure to locoweed during lactation initially resulted in decreased approach time; continuing exposure during lactation increased approach times (Fig. 2B), particularly on day 30.

Ewes intoxicated during gestation took a shorter path to reach the first lamb ($P=0.10$), but lactation exposure had no effect ($P>0.35$) (Table 1). There were no treatment interactions or treatment trial × interactions ($P>0.20$), nor were there any main effects of trial ($P>0.20$).

There were gestation ($P=0.09$) and lactation ($P=0.05$) effects, and a treatment × trial interaction ($P=0.08$) for percentage of time ewes spent away from their lambs after first contact during the 10-min tests. No significant differences were noted for test day 10 or day 30; however, on day 40 control ewes (CC) differed from all other treatment groups in the percentage of time they spent away from their lambs (Table 1).

4. Discussion

Serum AST activity in lactating ewes increased significantly during the first 15 days of locoweed feeding (James et al., 1970), then leveled off for the rest of the feeding period. When locoweed was withdrawn from the diets, only slight reductions in AST activity were noted. Continued locoweed-induced cellular damage, combined with lactational stress, may have contributed to the continued high AST activity even when locoweed was withdrawn from the diet. Apparent withdrawal stress has been noted in previous studies (Pfister et al., 1996; Stegelmeier, unpublished data). Serum swainsonine concentrations were sufficient to inhibit all cellular

Table 1
Distance traveled by ewes to first lamb (m), and percentage of time spent >1 m from own lamb by ewes during a 2-lamb choice test

Treatment group[a]	Distance traveled to first lamb[b] (m)	Percentage of time spent >1 m from own lamb (%)		
		Day[c] 10	Day[c] 30	Day[c] 40
Control:control (CC)	10.8	22	20	42[a]
Control:locoweed (CL)	11.3	27	12	8[b]
Locoweed:control (LC)	10.6	22	18	14[b]
Locoweed:locoweed (LL)	10.6	13	3	7[b]

Means within the same column with differing superscript letters (a and b) differ ($P < 0.1$).

[a] Control:control group received no locoweed; control:locoweed received locoweed during lactation; locoweed:control received locoweed during gestation; locoweed:locoweed received locoweed during both gestation and lactation.

[b] Meters traveled by ewe to reach first lamb (own or alien lamb). There was no treatment × trial interaction; there was a tendency towards a significant gestation effect ($P = 0.10$), but no lactation effect ($P > 0.35$).

[c] Number of days being fed locoweed. The values are percentage of time ewes spent away from own lambs after initial contact (10-min test).

α-mannosidase activity (Stegelmeier et al., 1995); thus, degree of intoxication was determined by length of exposure to the toxic compound. Subjectively, we evaluated ewes as being moderately intoxicated based on overt clinical signs such as slight intention tremors and proprioceptive deficits (Stegelmeier et al., 1995).

Lamb recognition was not adversely affected by locoweed. Recognition of lambs from a distance by dams is mainly determined by vision and hearing, and improves with dissimilarity of appearance, notably the head of the lamb (Poindron and Carrick, 1976; Alexander and Shillito, 1977; Searby and Jouventin, 2003). Locoweed intoxication apparently had no effect on the lamb recognition system mediated within the CNS (Ferreira et al., 2003).

Locoweed exposure during gestation, and an additional 10 days of exposure during lactation, decreased the approach times of ewes to the first lamb (either alien or own) during the 10-day test. Ewes exposed only for 10 days during lactation did not differ from controls. There are at least two possible explanations for this finding. First, locoweed exposure during gestation increased fetal expulsion times (Pfister et al., 2006a), and may have increased maternal responsiveness (Hinch et al., 1990). Second, CNS alterations (Van Kampen and James, 1970; Huxtable et al., 1982) may have resulted in maternal hypersensitivity (Maestrepieri et al., 1991). This is supported by the finding that CNS lesions, especially of the cerebellum, have been shown to accelerate mice responding in a maze compared to controls (Lalonde, 1987).

Ewes exposed to locoweed for 30 or 40 days during lactation (CL) and during lactation and gestation (LL) had slower approach times to the first lamb compared to controls (CC) and to ewes exposed only during gestation (LC). It is likely that the increased delay in reaching the lambs was due to CNS impairment, causing deficits in proprioception and intention tremors. Ewes showed clinical signs of intoxication, as they were easily startled and displayed intention tremors. Ewes intoxicated during gestation, however, took the shortest pathway to the first lamb. These results could be due to chance, or as we believe, may be a genuine reflection of the residual hyperkinetic effects of locoweed. Further, control ewes were very active after reaching their lambs, and often moved away from their lambs, whereas treated ewes (particularly CL and LL treatments) spent little time away from their lambs on day 40.

5. Conclusions

Our hypothesis that locoweed intoxication (at this dose and duration) would impair ewe discrimination of their lambs was rejected. The time for ewes to approach the first lamb was affected by locoweed history, with faster approach times initially, and slower times with increased lactation-related intoxication. Intoxicated ewes displayed a tendency to remain with their own lambs rather than exploring the arena as did controls. Neural and hormonal alterations induced by moderate locoweed intoxication apparently will not adversely affect lamb discrimination by ewes, and may heighten some aspects of maternal behavior, such as a ewe's propensity to remain near her lamb. Producers often cull intoxicated ewes for a variety of reasons,

including fear of abnormal maternal behavior, but the rapid resolution of histologic pathology (Pfister et al., 1996) and results from this study and others (Pfister et al., 1993, 2006a; K.E. Panter, unpublished data) suggest that culling intoxicated ewes may not be warranted if clinical signs are not severe.

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